Systematics of Bats of the Family Phyllostomidae Based on *RAG2* DNA Sequences

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Considerable disagreement exists concerning the phylogenetic relationships within the family Phyllostomidae (Wetterer et al., 2000). Numerous efforts have been made to partition the 53 genera (Wetterer et al., 2000) and more than 140 species (Koopman, 1993) into natural assemblages by using cranial, dental and skeletal (Miller, 1907; de la Torre, 1961; Walton and Walton, 1968; Slaughter, 1970; Phillips, 1971; Smith, 1976; Owen, 1982; Lim, 1993; Freeman, 2000; Wetterer et al., 2000), karyological (Baker, 1967; Gardner, 1977; Baker, 1979 and citations therein; Baker et al., 1981; Haiduk and Baker, 1982; Warner, 1983; Tucker, 1986; Tucker and Bickham, 1986), immunological (Gerber, 1968; Gerber and Leone, 1971; Straney, 1980; Baker et al., 1981; Honeycutt, 1981; Honeycutt et al., 1981; Arnold et al., 1982; Pierson, 1986; Honeycutt and Sarich, 1987;), soft anatomy (Forman, 1971; McDaniel, 1976; Griffiths, 1982; Hood and Smith, 1982, 1983; Gimenez, 1993; Gimenez et al., 1996; Wetterer et al., 2000), and ribosomal DNA restriction-site (Van Den Bussche 1991, 1992) data. A consensus among these diverse data has been that the vampires are monophyletic, but a phylogenetic tree or classification consistent with data from all studies has proven difficult to produce. As a result, past efforts to resolve the "true tree" have produced a morass of alternative hypotheses (which are reviewed by Wetterer et al., 2000, pp. 7-36).

In his classical work that has served as the benchmark for phyllostomid systematics, Miller (1907) recognized 51 genera in 7 subfamilies [Chilonycterinae (= Mormoopidae), Phyllostominae, Glossophaginae, Hemiderminae (= Carolliinae), Sturnirinae, Stenoderminae, Phyllonycterinae]. He included in the family the species currently recognized as members of Mormoopidae, but excluded the genera of vampire bats, placing them in a separate family (Desmodontidae). Subsequent work by Forman et al. (1968) documented that the vampire bats were part of the phyllostomid radiation. Smith (1972) recognized Mormoopidae (Miller's subfamily Chilonycterinae) as distinct from Phyllostomidae.

With the inclusion of the vampire bats and the exclusion of mormoopids, classifications of phyllostomids thereafter became stable with respect to the naturalness or monophyly of the family. However, the number of genera and subfamilies recognized has varied considerably. For example, Baker et al. (1989) recognized 44 genera in three subfamilies (Desmodontinae, Vampyrinae, Phyllostominae), Koopman (1993) recognized 49 genera in eight subfamilies (Phyllostominae, Lonchophyllinae, Brachyphyllinae, Phyllonycterinae, Glossophaginae, Carolliinae, Stenodermatinae, Desmodontinae), McKenna and Bell (1997) recognized 48 genera in four subfamilies (Desmodontinae, Glossophaginae, Phyllostominae, Stenodermatinae) and Nowak (1999) followed Van Den Bussche (1992) in recognizing 52 genera in five subfamilies (Desmodontinae, Macrotinae, Micronycterinae, Vampyrinae and Phyllostominae). The latest and most comprehensive effort (Wetterer et al., 2000) recognized 53 genera in seven subfamilies (Desmodontinae, Brachyphyllinae, Glossophaginae, Phyllonycterinae, Phyllostominae, Stenodermatinae, Carolliinae) in a total evidence synthesis of extensive new and previously published data; however, this synthesis excluded autosomal G-band chromosomal data because Wetterer et al. (2000, p. 36) concluded *a priori* that primitive and derived character states for these data had not been developed properly. Their "total evidence" provided little support, based on bootstrap and decay analysis, for many clades in their tree. About 70% of the clades collapsed in a tree only 1 step longer (decay = 1) and 50% of the clades were supported by bootstrap values < 50%. Some of their most critical conclusions for monophyly had weak bootstrap (bs) and decay (d) support (i.e., Phyllostominae, bs = 40, d = 1; Carolliinae, bs = 33, d = 1; Stenodermatinae, bs = 54, d = 1). Wetterer et al. (2000) proposed that their data and hypotheses provide a starting point for new and productive investigations of phyllostomid relationships and evolution. Therefore, we use their tree (p. 134) and the tree from Baker et al. (1989) as the primary reference for comparison with our resulting gene tree.

A robust tree for Phyllostomidae would provide substantial information. The morphological variability of this family of bats provides a unique opportunity to study the evolution of new genera and new feeding strategies, and to develop hypotheses concerning the primitive character states of ancestral stocks for the lineages that evolved into new feeding niches (Freeman, 2000; Ferrarezi and Gimenez, 1996; Wetterer et al., 2000). This study is part of a long-term effort to provide a phylogenetic tree against which the remarkable evolutionary radiation of phyllostomids can be better understood. Our initial efforts at using DNA sequence data involved the mitochondrial cytochrome-*b* gene. The rate of evolution of the cytochrome-*b* gene within Phyllostomidae was appropriate for elucidating relationships within genera (Van Den Bussche and Baker, 1993; Van Den Bussche et al., 1993, 1998; Baker et al., 1994; Wright et al., 1999). But saturation of some types of mutations complicate resolution at higher taxonomic levels. In this paper, we examined DNA sequence data from the Recombination-Activating Gene-2 (*RAG2*) to infer the deep-branching patterns within this complex of bats.

RAG proteins are encoded by two tandemly paired genes (*RAG1* and *RAG2*) that are uninterrupted by introns (in tetrapods) and located within 8 kb of each other in the nuclear genome (Fig. 1). Human *RAG1* and *RAG2* proteins are 1,043 and 527 amino acids, respectively (Shatz et al., 1989; Oettiger et al., 1990). These genes apparently arose as a transposon in the ancestor of jawed vertebrates (gnathostomes), and their products still retain some transposition activity *in vitro* (Agrawal et al., 1998; Hiom et al., 1998; Plasterk, 1998). However, after their insertion into the gnathostome nuclear genome, the *RAG* proteins assumed a significant function in the immune system. The genes are active in lymphocytes where their protein products catalyze V(D)J recombination, the process by which immunoglobulin genes are assembled (Schatz et al., 1989; Oettinger et al., 1990; McBlane et al., 1995; Melek et al., 1998; Plasterk, 1998; Akamatsu and Oettinger, 1998; Mo et al., 1999; Steen et al., 1999; Swanson and Desiderio, 1999). Because of the immunological role played by the *RAG2* protein, sequence variation in the gene should provide an estimate of phyllostomid evolution that is seemingly largely uncorrelated with the extensive morphological adaptations within this family.

MATERIALS AND METHODS

Specimens and DNA Preparation.—Tissue samples were obtained from collections at the Natural Science Research Laboratory of the Museum of Texas Tech University, Museum of Southwestern Biology at the University of New Mexico, National Museum of Peru, the American Museum of Natural History and the Royal Ontario Museum (Table 1). Each sample used in the study is associated with a voucher specimen (Ruedas et al., 2000) deposited in a mammal collection at Texas Tech University, American Museum of Natural History, the Museum of Southwestern Biology at the University of New Mexico, Carnegie Museum of Natural History, Universidad Nacional Autónoma de Mexico, Muséum National d'Histoire Naturalle, National Museum of Natural History, Texas Cooperative Wildlife Collection, Universidad Autónoma Metropolitana-Iztapalapa, Royal Ontario Museum or the National Museum of Peru. Total genomic DNA was isolated following the procedures of Longmire et al. (1997).

Gene amplification and Sequencing.—We amplified via the polymerase chain reaction (PCR) a portion of the *RAG2* protein coding sequence from total genomic DNA preparations. A schematic of our primer positions is shown in Figure 1 and primers are described in Table 2. A segment of approximately 1.4 kb was amplified using primer RAG2-F1 or RAG2-F1B paired with RAG2-R2 (Table 2 and Fig. 1). For some species two overlapping segments were amplified using primers RAG2-F1 and RAG2-R1 for one fragment and primers RAG2-F2 and RAG2-R2 for the other. Primers RAG2-F1 and RAG2-F1B anneal 122 and 138 bp downstream of the first base of the initiation codon. Primer RAG2-R2 anneals 39 bp upstream of the stop codon. PCR reaction mixtures contained 200 μM of each dNTP, 1.5 mM $MgCl_2$, 2 ng/μl of each primer, 5-10 ng/μl of template DNA, and 0.05 units/μl of Promega Taq DNA polymerase, in a 1X solution of Promega Taq polymerase buffer. Reaction conditions were as follows: initial denaturation, 2 min

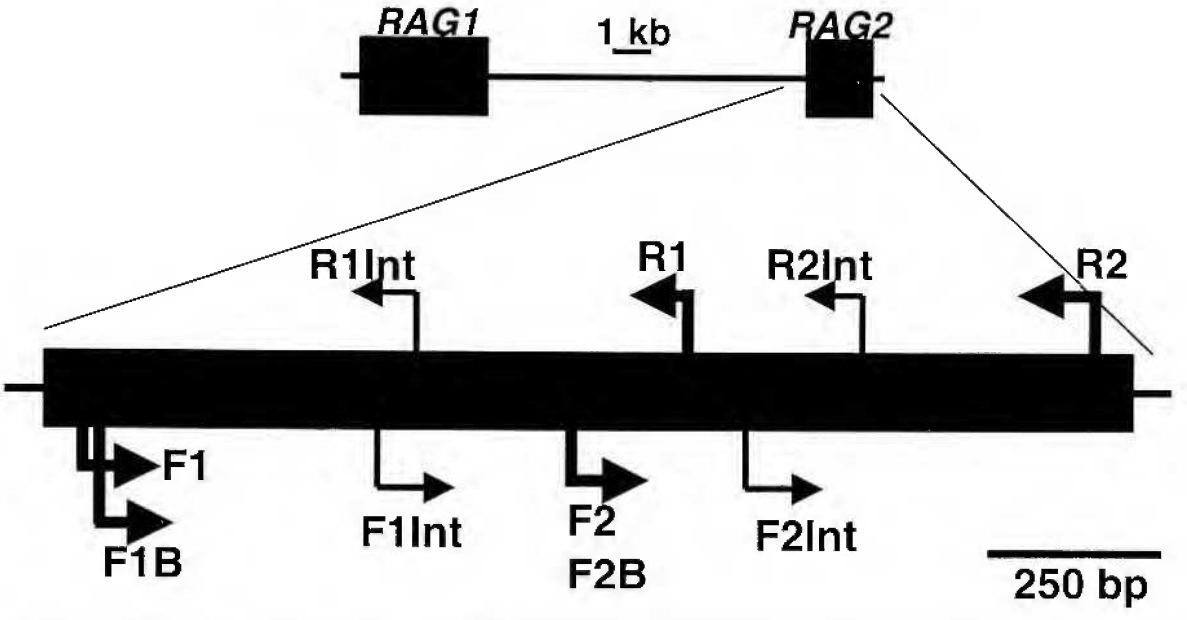

Figure 1. Diagrammatic representation of the *RAG* genes, showing primer-annealing sites. The upper diagram shows the relationship of both *RAG* genes, with the coding regions represented by black boxes. The lower figure shows the primer annealing sites in the *RAG2* gene. PCR primers are represented by bold arrows. The "*RAG2*" prefix is omitted from the primer names.

95°C, followed by 35 cycles of (denaturation, 30 sec, 95°C; annealing, 30 sec, 65°C; polymerization, 2 min, 72°C), with a 10 min final extension at 72°C. If this thermal profile failed to produce an amplification product, we reduced the annealing temperature to 60°C, 55°C, or 50°C as needed to produce positive results. These methods are modified from Bickham et al. (1996).

We purified PCR products using a QIAquick PCR purification kit from QIAGEN. Sequencing reactions were performed using Terminator Cycle Sequencing Ready Reactions (either dRhodamine or BigDye™) from ABI, and were purified by ethanol precipitation. PCR primers also were used for sequencing, along with internal primers (Table 2 and Fig. 1). Both strands were sequenced and samples were run on an ABI 310 Genetic Analyzer, and final sequences were assembled using Sequencher™, version 3.1.1 of Gene Codes Corporation.

Data Analysis.—DNA sequences were aligned using Clustal W (Thompson et al., 1994). Sequence data were treated as unordered, discrete characters (G, A, T, C) and polarity of character-state changes was established by designating representatives of Emballonuridae, Furipteridae, Noctilionidae, and Mormoopidae as outgroup taxa (see specimens examined). Phylogenetic analyses were performed using PAUP*4.04a (Swofford 2000). To evaluate whether these data contained phylogenetic information, the Random Tree option of PAUP* was used to evaluate the distribution of 100,000 random trees using the g_1-statistic (Hillis, 1991; Huelsenbeck, 1991; Hillis and Huelsenbeck, 1992). Parsimony analyses were conducted utilizing equal weights for all characters and the successive weighting approach (Farris, 1969; Carpenter, 1994) via the rescale option in PAUP*4.04a. Because sequences from 66 individuals (64 taxa) were included, exhaustive and branch and bound searches for the most parsimonious tree(s) would have required a prohibitive amount of computing time (Swofford and Olsen 1990). Therefore, we conducted heuristic searches with 25 random additions of input taxa and tree bisection reconnection (TBR) branch swapping (Swofford and Olsen 1990). Stability or accuracy of inferred topologies was assessed via bootstrap analysis (Felsenstein, 1985) of 200 iterations with 25 random additions of input taxa and TBR for each iteration.

*Table 1. List of specimens sequenced for this study. Specimens were collected from natural populations and voucher specimens are housed at American Museum of Natural History (AMNH), Carnegie Museum of Natural History (CMNH), Universidad Nacional Autónoma de Mexico, Mexico City (IB UNAM), Muséum National d'Histoire Naturalle, Paris (MNHN), Museum of Southwestern Biology, University of New Mexico (MSB), Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima (MUSM), National Museum of Natural History (NMNH), Royal Ontario Museum, Toronto (ROM), Museum of Texas Tech University (TTU), Texas Cooperative Wildlife Collection, Texas A&M University (TCWC), or Universidad Autónoma Metropolitana-Iztapalapa, Mexico City (UAM-I). ***Location of voucher specimen undetermined.*

Taxon	Sex	TK Number	Locality	Museum Catalog Number
Ametrida centurio	M	TK 18810	French Guyana: Paracou: near Sinnamary	AMNH 267279
Anoura geoffroyi	M	TK 34863	El Salvador: Santa Ana: Parque Nacional Montechristo, Los Planes	TTU 62405
Artibeus concolor	F	TK 10378	Surname: Commewijne: Nieuwe Gron Plantation, 5°53'N, 54°54'W	CMNH 63792
Artibeus hirsutus	F	TK 11492	México: Sonora: 5 mi NW San Carlos	MSB 54922
Ardops nichollsi	M	TK 15602	Dominica: St. Joseph Parish: Clarke Hall Estates	TTU 31357
Ariteus flavescens	M	TK 27696	Jamaica: St. Anne's Parish: Circle B Plantation, 2 km SW Priory	TTU 45291
Brachyphylla cavernarum	M	TK 15630	Dominica: St. Paul Parish: Stinking Hole Cave, above Cochrane	TTU 31435
Carollia brevicauda	M	TK 19316	Venezuela: Barinas, 7 km NW Barinitas	CMNH 78409
Centurio senex	M	TK 13110	México: Veracruz: Ojo de Agua, Rio de Atoyac, 14 km N, 22 km E Córdoba	CMNH 55731
Chiroderma villosum	M	TK 7978	Costa Rica: San Jose: 12.3 mi SSE San Isidro	TTU 34311
Choeroniscus godmani	M	TK 40021	El Salvador: San Miguel: Hacienda Lechera "El Cañal"	TTU 62406
Choernycteris mexicana	M	TK 20501	México: San Luis Potosí: 15 mi S, 1 mi E Huizache	TTU 36118
Chrotopterus auritus	M	TK 17104	Suriname: Saramacca, Raleigh Falls, 4°44' N, 56°12'W	CMNH 68638
Dermanura cinerea	F	TK 18790	French Guyana: Paracou: near Sinnamary	AMNH 267197?MNHN 1995.1110
Desmodus rotundus	M	TK 4764	México: Guerrero: 24.1 mi N Rio La Unión on Hwy 200	TTU 35582?IB-UNAM
Diaemus youngi	F	TK 34625	El Salvador: La Paz: 1 mi N La Herradura	TTU 62792
Diclidurus albus	M	TK 19549	México: Jalisco: 12 km SW Chamela	TTU 36438
Diphylla ecaudata	F	TK 13514	México: Yucatán: 1 km N Mérida	***
Ectophylla alba		TK 16395	Costa Rica: Cano Palma Biological Station, 7 km NNW Tortuguerro	ROM 108296
Enchisthenes hartii	M	TK 22690	Perú: Huanuco: 11 km N, 6 km S Tingo Maria, 9°12'S, 75°56'W	CMNH 98710
Erophylla sezekorni	M	TK 9416	Jamaica: Hanover Parish: Flint River, 1.5 mi E Sandy Bay	CMNH 44507
Furipterus horrens	F	TK 17149	Suriname: Saramacca: Voltzberg, 4°40'N, 56°12'W	CMNH 68439
Glossophaga soricina	M	TK15311	Venezuela: Miranda: Guatopo National Park, Santa Crucita campground	TTU 33324
Glyphonycteris daviesi			Equador: Onkonegar	ROM 105495
Glyphonycteris sylvestris	F	TK 10453	Suriname: Brokopondo: 2 km W, 8 km S Brownsweg, 4°55'N, 55°11'W	CMNH 63593
Hylonycteris underwoodi	M	TK 20540	México: Tabasco: 3 km E Teapa, Grutas de Cocona	TTU 36152
Lampronycteris brachyotis	M	TK 25238	Trinidad: Mayaro: Guayagayare	TCWC 25238
Leptonycteris curasoae	M	TK 45108	México: Michoacán: 1 km N Playa Azul	UAM-I (not cataloged)
Lionycteris spurelli	M	TK 10164	Suriname: Nickerie: 1 km S, 3.5 km E Sipaliwini Airstrip	CMNH 63712
Lonchophylla thomasi	M	TK 17177	Suriname: Saramacca: Voltzberg, 4°40'N, 56°12'W	CMNH 68779
Lonchorhina aurita	M	TK 20560	México: Chiapas: 8.2 mi SE, 2 mi E Tonala, Rio Ocuilapa	TTU 36531

Table 1. cont.

Macrophyllum macrophyllum	F	TK19119	Venezuela: Bolivar: 8 km S, 5 km E El Manteco	CMNH 78289
Macrotus californicus	M	TK 28962	USA: Arizona: Pinal Co.	***
Macrotus waterhousii	F	TK 28535	México: D.F.: 34 km S Cd. de México, La Cima	TTU 45025?IB-UNAM
Macrotus waterhousii	M	TK 32030	Cuba: Guantánamo: Guantanamo Bay Naval Base	TTU 52481
Mesophylla macconnelli	F	TK 55316	Perú: Cusco: La Convención, Camisea, Pagoreni	NMNH
Micronycteris hirsuta	F	TK 25041	Trinidad: St. George: 4 km N Arima, Simla Research Center	CMNH 97177
Micronycteris hirsuta	F	TK 17259	Suriname: Para: Zanderij, 5°27'N, 55°12'W	CMNH 68388
Micronycteris megalotis	M	TK 18785	French Guyana: Paracou: near Sinnamary	AMNH 268090
Micronycteris minuta	M	TK 17877	Suriname: Nickerie: Sipaliwini Airstrip, 2°2'N, 56°7'W	CMNH 77111
Micronycteris schmidtorum		TK 70447	Perú: Camisea	MUSM 13737
Mimon crenulatum	M	TK 15121	Venezuela: Guarico: 45 km S Calabozo	TTU 33287
Monophyllus redmani	M	TK 27694	Jamaica: St. Ann's Parish: 2 km SW Priory, Circle B Plantations	TTU 45274
Mormoops megalophylla	M	TK 48184	USA: Texas: Presidio Co.: Big Bend Ranch State Natural Area	TTU 68296
Musonycteris harrisoni	M	TK 43171	México: Michoacán: Mun. Aguila, 6 km N Maruata	UAM-I (not cataloged)
Noctilio albiventris	M	TK 46004	Perú: Loreto: Quebrada: Agas Negras Cocha Oraida	MUSM
Noctilio leporinus	M	TK 18701	Grenadines: Carriacou Island: Craigston Estate	CMNH 63177
Phyllonycteris aphylla	M	TK 9280	Jamaica: Westmoreland Parish: Bluefields	CMNH 44536
Phyllostomus hastatus	M	TK 19243	Venezuela: Bolivar: 8 km W El Manteco	CMNH 78333
Phylloderma stenops	M	TK 10201	Suriname: Saramacca: Raleigh Falls, 4°44'N, 56°12'W	CMNH 63614
Platyrrhinus helleri	M	TK 17142	Suriname: Saramacca: Voltzberg, 4°40'N, 56°12'W	CMNH 69026
Pternotous davyi	F	TK 25127	Trinidad: Nariva: San Rafael Ward, Arena Reserve (3 mi S, 3 mi W Cumuto)	TTU 43938
Pygoderma bilabiatum	F	TK 12682	Bolivia: Santa Cruz Dept: San Rafael do Amboro	MSB 55894
Rhinophylla pumilio	F	TK 18825	French Guyana: Paracou: near Sinnamary	AMNH 267158
Saccopteryx bilineata	M	TK 18839	French Guyana: Paracou: near Sinnamary	AMNH 267057
Sphaeronycteris toxophyllum	F	TK 55329	Perú: Amnihuari	MUSM
Stenoderma rufum	M	TK 21790	Puerto Rico: Rio Grande: El Verde Field Station, 18°19'8"N, 65°49'12"W	TTU 46374
Sturnira lilium	M	TK 25163	Tobago: St. Patrick Co.: Grange	TTU 44085
Tonatia brasiliense	F	TK 18834	French Guyana: Paracou: near Sinnamary	AMNH 267103
Trachops cirrhosus	M	TK 18829	French Guyana: Paracou: near Sinnamary	AMNH 267129
Trinycteris nicefori	M	TK 15189	Venezuela: Guarico	***
Uroderma bilobatum	M	TK 34926	El Salvador: La Paz: Zacatecoluca, Hacienda Escuintla	TTU 62490
Vampyressa bidens	F	TK 55322	Perú: Cusco: La Convencion, Camisea, Pagoreni	MUSM
Vampyressa pusilla	F	TK 70533	Perú: Cusco: La Convencion, Camisea, Pagoreni	MUSM
Vampyrodes caraccioli	F	TK 25083	Trinidad: St. George: 4 km N Arima, Simla Research Center	CMNH 94707
Vampyrum spectrum	M	TK 40370	Honduras: Atlántida: Lancitilla	TTU 61070

Table 2. Sequences of primers used for amplifications and sequencing of RAG2. All sequences are given to 5' to 3'.

RAG2-F1	GGCYGGCCCAARAGATCCTG
RAG2-F1Int	GRACAGTCGAGGGAARAGCATGG
RAG2-F1B	ATCCTGCCCCACTGGAGTTTTC
RAG2-F2	TTTGTTATTGTTGGTGGCTATCAG
RAG2-F2B	GTTRTTGTRGTTGGCTATCA
RAG2-F2Int	GGAYTCCACTCCCTTTGAAGA
RAG2-R1	AACYTGYTTATTGTCTCCTGGTATGC
RAG2-R1Int	GGGGCAGGCASTCAGCTAC
RAG2-R2	GRAAGGATTTCTTGGCAGGAGT
RAG2-R2Int	GCAGCAWGTAATCCAGTAGC

RESULTS

A segment of approximately 1.4 kb of the *RAG2* gene was sequenced for 64 taxa representing 5 microchiropteran families, and these sequences have been deposited in GenBank (Accession numbers AF316430 – AF316495). Sequence alignment resulted in 1,363 aligned sites, of which 346 (25.4%) were parsimony-informative. Fifty-nine (17.1%) of the parsimony informative sites occurred at first positions, 31 (9.0%) at second positions, and 256 (74.0%) at third positions of codons. The g_1-statistic of 100,000 random trees (g_1 = -0.628) indicated that the distribution of tree lengths was highly skewed to the left, suggesting a high probability for the correct topology being the most-parsimonious tree or a tree a few steps longer (Hillis, 1991; Huelsenbeck, 1991; Hillis and Huelsenbeck, 1992—for criticisms of this statistic see Kallersjo et al., 1992).

Unweighted parsimony analysis resulted in 3,456 equally parsimonious trees of 1,122 steps [consistency index (CI) = 0.4180; retention index (RI) = 0.6643] and most clades received moderate to strong bootstrap support (Fig. 2). Parsimony analysis using the successive weighting approach resulted in 48 most-parsimonious trees of 257.44 steps (CI = 0.6261; RI = 0.8323) and most clades received moderate to strong bootstrap support (Fig. 2b).

DISCUSSION

RAG2 is a slowly evolving nuclear gene that provides a new view into the evolutionary history within the family Phyllostomidae. *RAG2* is particularly useful in phyllostomids because its function in immunological response probably is not linked to morphological features that often are the diagnostic criteria for higher-category classifications. The magnitude of morphological adaptation to feeding strategies has resulted in an array of phenotypes within Phyllostomidae that are modified sufficiently to mask basal phylogenetic relationships. For example, all studies agree that the three genera of vampire bats are monophyletic; however, no robust sister-taxon hypothesis has been developed to explain the origin of sanguivory. The situation with the basal relationship of the vampires to the rest of the family is extreme but not unique, which probably accounts for the large number of competing hypotheses that have been advanced to explain the deep-branching relationships of phyllostomids. Our conclusion is that gene trees should be explored as a means to avoid complications (Griffiths, 1982; Baker et al., 1989; Wetterer et al., 2000) that have hampered all previous classifications. Any lineage documented by shared-derived morphological features (Slaughter, 1970; Griffiths, 1982; Baker et al., 1989; Freeman, 2000; Wetterer et al., 2000) and monophyly within the gene tree would have considerable support as a natural assemblage. Therefore, we compare our *RAG2*

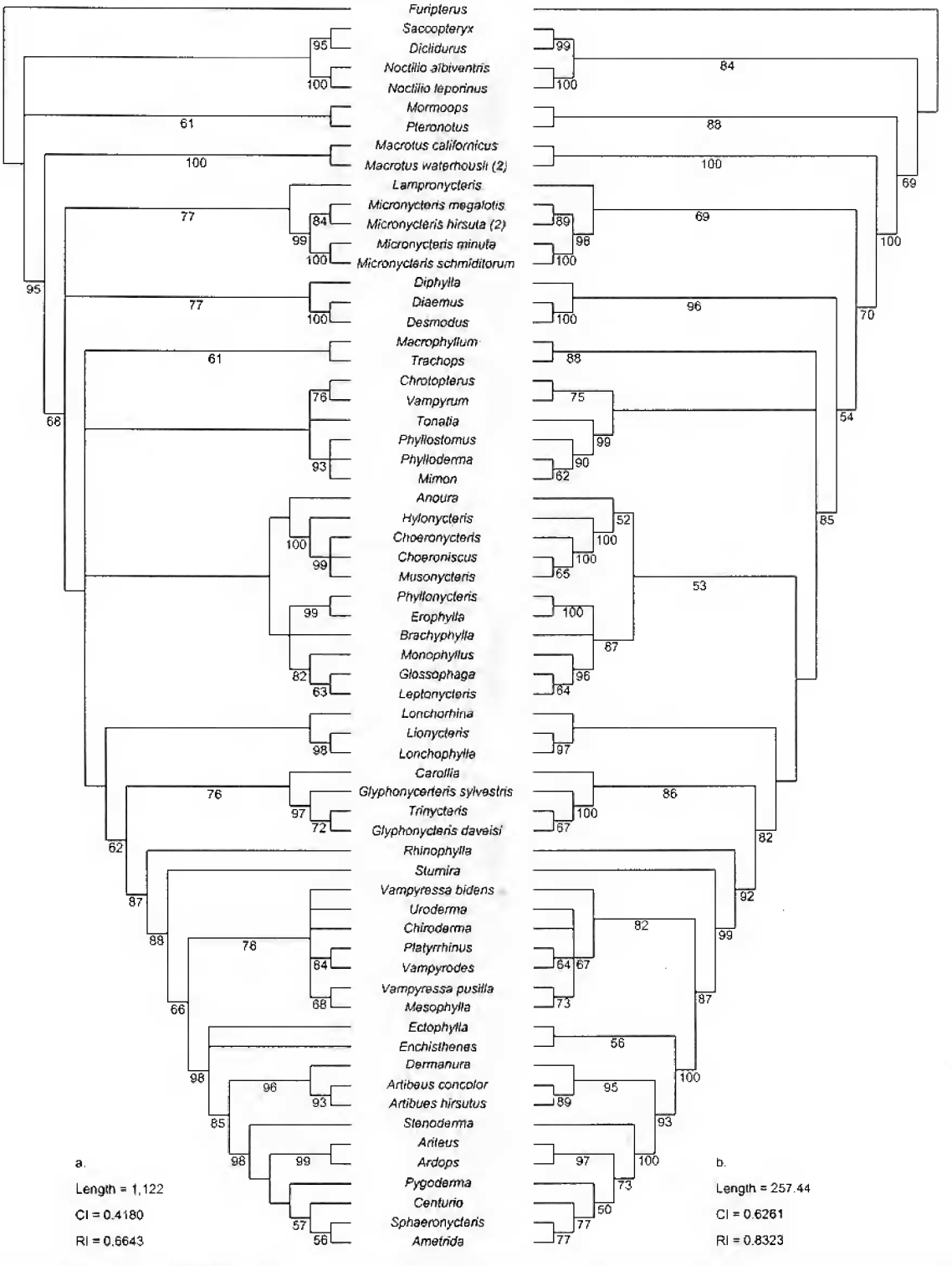

Figure 2. (a.) Topology of strict consensus of 3,456 most parsimonious trees resulting from an unweighted parsimony analysis. Numbers below branches indicate percentage of 200 bootstrap iterations with 25 random additions of input taxa and TBR branch swappings that each clade was detected. (b.) Topology of strict consensus of 48 equally parsimonious trees of 257.44 steps resulting from heuristic searches with 25 TBR and successive weighting based on the rescaled consistency index. Numbers along branches indicate the percentage of 200 bootstrap iterations with 25 TBR that each clade was detected. For both trees (a and b), bootstrap values are shown only for those clades detected in 50% or more of iterations.

gene tree to previous trees and classifications, specifically to those of Baker et al. (1989; Fig. 3) and Wetterer et al. (2000; Fig. 4) who synthesized diverse data sets and classifications for the Phyllostomidae.

To test for equivalence between our strict-consensus tree (Fig. 2) and the topologies proposed by Baker et al. (1989) and Wetterer et al. (2000), we constrained the *RAG2* trees to document their branching patterns. We tested these competing topologies using the Kishino-Hasegawa (1989), Templeton (1983), and winning-sites (Prager and Wilson, 1988) tests. The Baker et al. (1989) and Wetterer et al. (2000) constrained trees were 66 and 561 steps longer, respectively, than our strict-consensus tree based on an unweighted analysis (Fig. 2a) and all three tests indicated significant differences between these competing hypotheses and our resultant phylogeny ($P < 0.0001$). The higher values (steps longer) in the comparison of Wetterer et al.'s tree to Baker et al.'s tree are more of a function of the detail of Wetterer et al.'s tree rather than a high level of mismatch.

In the classification proposed by Baker et al. (1989) the position of *Macrotus* was uncertain and was placed *incertae sedis* based in part on the conclusion that the karyotype of *Macrotus waterhousi* is like that proposed as primitive for the family. The *RAG2* gene tree sets *Macrotus* as basal for the phyllostomids, which is compatible with the chromosomal conclusions of Patton and Baker (1978). The situation with *Micronycteris* is more complex, as the *RAG2* gene tree divides this genus (*sensu* Koopman, 1993) into two divergent clades at different places in the tree (Fig. 2). Some major points of disagreement with the Baker et al. (1989) classification (Fig. 3) include the diphyly of Glossophaginae, with *Lonchophylla* and *Lionycteris* representing an independent origin of nectar feeding. A similar diphyly hypothesis was proposed by Winkelmann (1971) and Griffiths (1982). Additionally, in the *RAG2* data, there is no support for Vampyrinae as recognized by Baker et al. (1989). Finally, Baker et al. (1989) recognized the Phyllostomini, for which monophyly is questioned by the *RAG2* data because *Lonchorhina* is not associated with that group.

A comparison of the *RAG2* gene tree (Fig. 2b) to the Wetterer et al. (2000) heuristic search tree using all 150 characters for all 63 taxa (Fig. 4) also reveals several incongruencies. For example, in the tree produced by Wetterer et al. (2000), the Micronycterini consisted of the previously recognized genus *Micronycteris* (*sensu* Koopman, 1993) with the genus *Macrotus* nested as a central taxon (Fig. 4). In our tree, *Macrotus* (Fig. 2b) is basal even to the vampire bats, forming the first clade of the Phyllostomidae. *Micronycteris* is further subdivided, with five species (*schmidtorum*, *minuta*, *hirsuta*, *megalotis*, and *brachyotis*) forming one clade that is the second branch after *Macrotus* (Fig. 2b). Three species of *Micronycteris* (*sensu* Koopman, 1993), (*sylvestris*, *nicefori*, and *daviesi*) are sister to *Carollia*, and this group forms a clade that is basal to Stenodermatinae which together are successively sister to *Rhinophylla*. *Micronycteris* (sensu Koopman) was divided into five genera by Simmons and Voss (1998) and we follow that arrangement.

The implications of the *RAG2* tree are not trivial because the karyotype of *Macrotus waterhousii* was concluded to be primitive for the family based on a cladistical analysis of chromosomal data using *Noctilio*, *Mormoops*, and *Pteronotus* as outgroups (Patton and Baker, 1978). If indeed the karyotype of *Macrotus* is primitive for the family, the placement of *Macrotus* within *Micronycteris* (*sensu* Koopman, 1993) would be problematic for the chromosomal data in the tree generated by Wetterer et al. (2000). Wetterer et al. (2000) addressed this issue by concluding that the "chromosomal structure of *Macrotus* may have converged on the state seen in *Pteronotus* and *Noctilio*... this possibility affects the interpretation of all chromosomal data reported thus far as *Macrotus* was the primitive reference taxon for most studies." Further, they used this explanation of chromosomal data to explain other areas in their tree that were incongruent with the chromosomal data (i.e. the potential chromosomal synapomorphy that unites *Glossophaga*, *Leptonycteris*, *Brachyphylla*, *Erophylla*, and *Phyllonycteris* – Baker and Bass, 1979; Haiduk and Baker, 1982). In our gene tree, *Brachyphylla*, *Phyllonycteris*, *Glossophaga*, and *Leptonycteris* form a clade that is compatible with the chromosomal data just as the position of *Macrotus* to the remainder of the family is compatible with the proposed primitive character-states for *Macrotus waterhousii*. Our data suggest that Phyllostominae is not monophyletic, contrary to Wetterer et al. (2000) for two reasons. First, *Macrotus* and *Micronycteris* (*Micronycteris*, *Lampronycteris*, and possibly *Neonycteris*) are basal to the vampires and all other

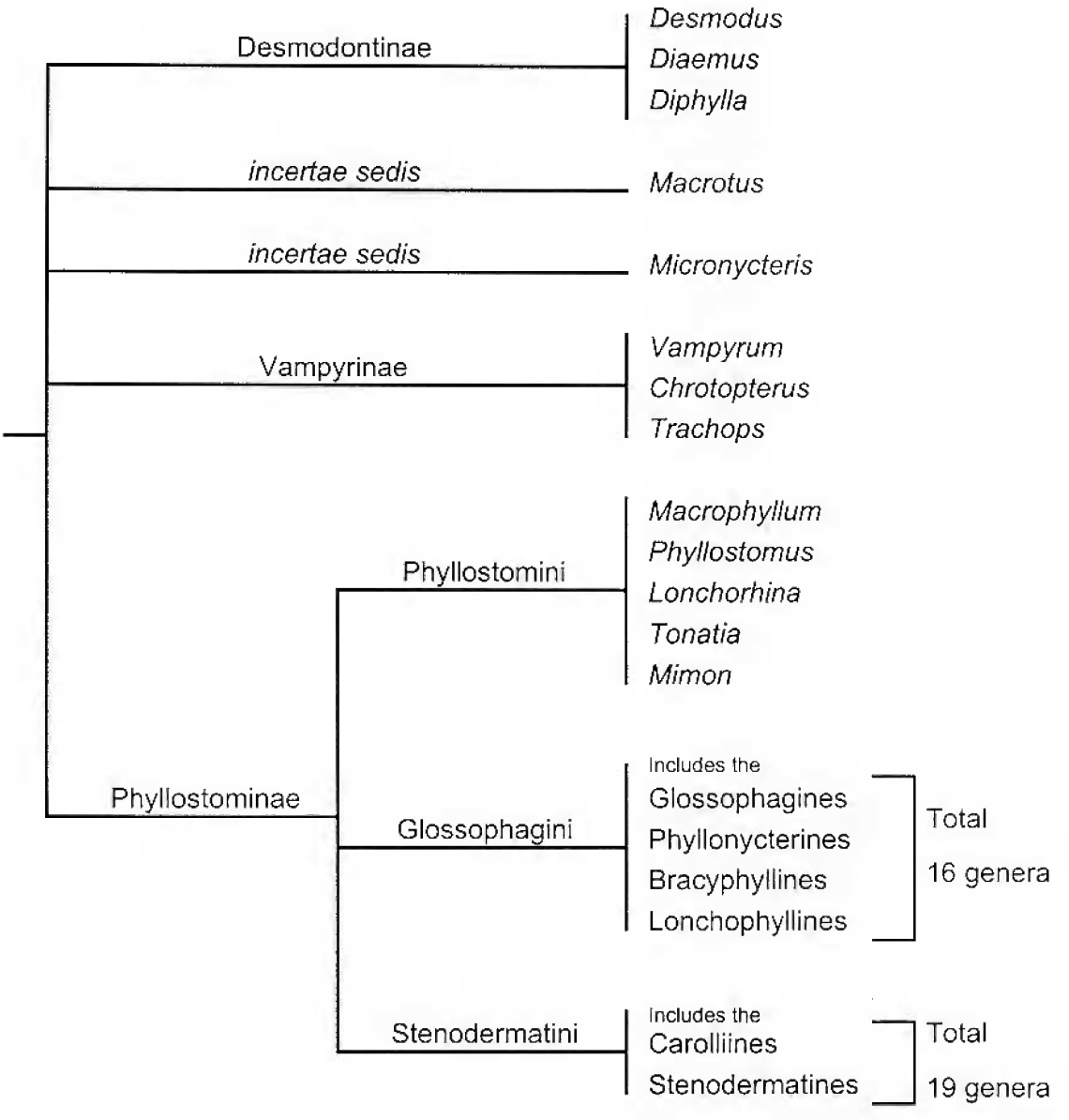

Figure 3. Baker et al.'s consensus tree (1989; redrawn from their Fig. 2) based on morphology, karyology and immunology.

phyllostomid genera. Second, other genera in the Phyllostominae (*sensu* Wetterer et al., 2000) *Lonchorhina* and *Glyphonycteris daviesi*, *G. sylvestris* and *Trinycteris nicefori* along with *Carollia* and the Stenodermatinae to the exclusion of *Vampyrum*, *Chrotopterus*, *Mimon*, *Phylloderma*, *Phyllostomus*, *Tonatia*, *Macrophyllum*, and *Trachops* suggest that Phyllostominae is not monophyletic.

There are several other places where the *RAG2* gene tree contradicts that of Wetterer et al. (2000). *Carollia* and *Rhinophylla* (the only two genera in Carolliinae) do not form a monophyletic group in the strict-consensus of our most-parsimonious trees. *Lonchophylla* and *Lionycteris*, members of the Glossophaginae (*sensu* Wetterer et al., 2000), do not share a common ancestor with the remainder of their

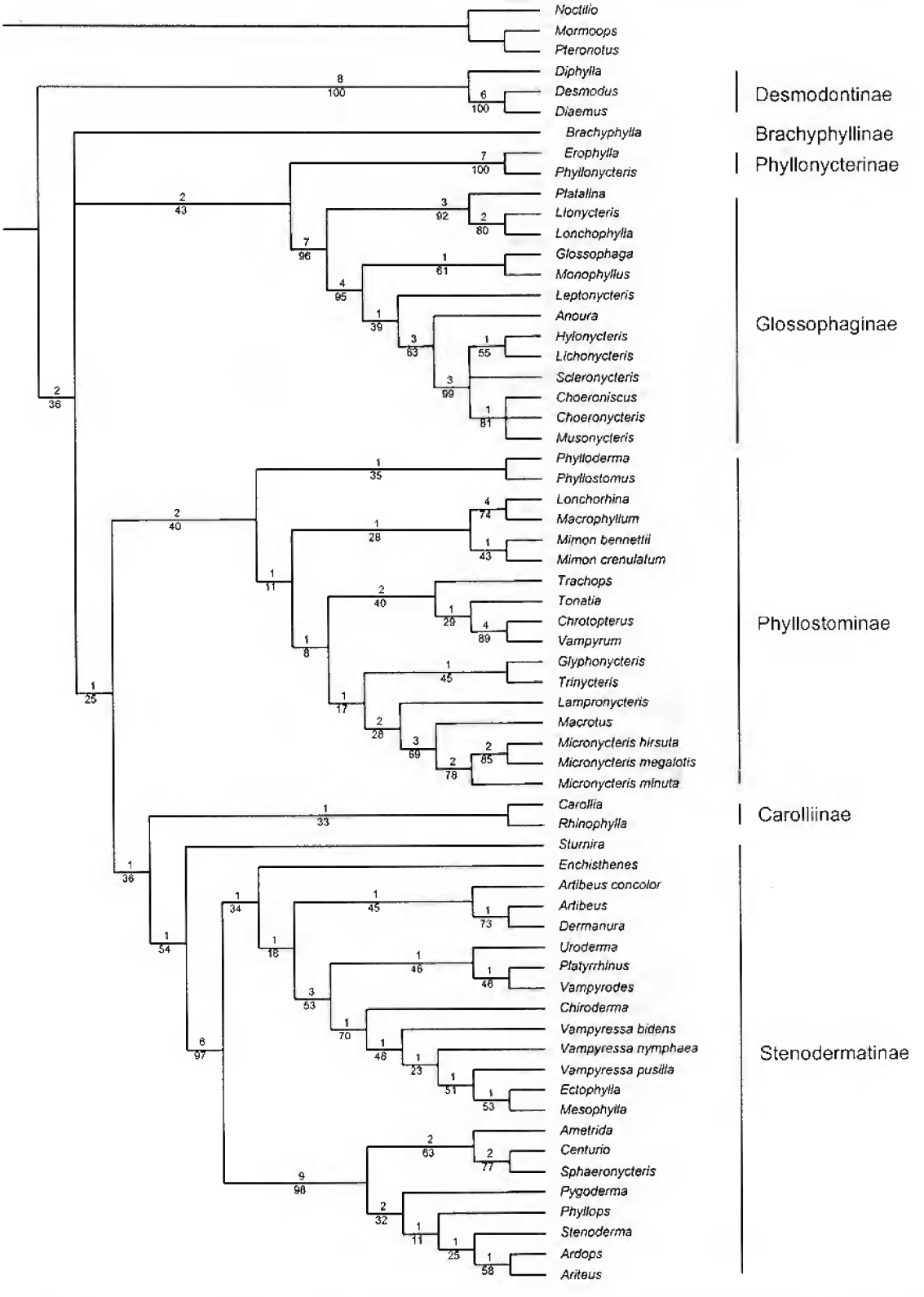

Figure 4. Wetterer et al.'s total evidence tree (2000; redrawn from their Fig. 49, with nomenclature adjusted to conform to Fig. 2) based on the results of a heuristic search using all 150 characters for all 63 taxa. Numbers appearing above the line are decay values; below the line are bootstrap values. To the right is their subfamilial classificiation.

glossophagine taxa. The *RAG2* gene data strongly support the common ancestry of *Mesophylla* and *Vampyressa pusilla* to the exclusion of *Ectophylla*. Wetterer et al. (2000) concluded, based on essentially no bootstrap or decay support, that *Ectophylla* and *Mesophylla* were congeneric. *RAG2* moderately supports that *Ectophylla* and *Enchisthenes* shared a common ancestor after diverging from a clade with *Artibeus*, *Dermanura* and the short-faced bats (Fig. 2). This supports the conclusions of Van Den Bussche et al. (1993, 1998), Pumo et al. (1996) and Tandler et al. (1997) that *Enchisthenes* is not a member of the *Dermanura*-clade, a position supported by the analysis of Wetterer et al. (2000).

Although differences exist between our tree and the topology presented by Wetterer et al. (2000), *RAG2* sequence data do support some relationships proposed by Wetterer et al. (2000): 1) topology of *Micronycteris* [(*sensu* Simmons and Voss, 1998): *M. hirsuta* + *M. megalotis* + *M. minuta*]; 2) monophyly of Desmodontinae; 3) topology of *Anoura* + *Hylonycteris* + *Choeroniscus* + *Choeronycteris* + *Musonycteris*; 4) monophyly of *Lonchophylla* and *Lionycteris*; 5) monophyly of stenodermatines; and 6) monophyly of the short-faced bats (*Ametrida*, *Ardops*, *Ariteus*, *Centurio*, *Phyllops*, *Pygoderma*, *Sphaeronycteris*, and *Stenoderma*).

Although trees produced from *RAG2* DNA sequences are significantly different from topologies and resultant classifications proposed by Baker et al. (1989) and Wetterer et al. (2000), we feel it is inappropriate to suggest major taxonomic changes based on the analysis of a single gene tree. Clearly, further studies are necessary to provide resolution to these contradictory hypotheses. What is needed now are data from additional unlinked genes to see if these incongruences between the *RAG2* gene tree and other proposed phylogenies simply add to the morass of systematic hypotheses or if DNA sequence data will help solve this complex systematic riddle.

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